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Protection by Steroids against Acute $HgCl_2$ Poisoning

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Experiments on rats indicate that the fatal renal damage normally produced by acute $HgCl_2$ intoxication is more effectively prevented by thioacetyl containing steroids (e.g., spironolactone, spiroxasone or emdabol) than by inorganic sodium thioacetate. Steroids posssesing sulfur in forms other than thioacetyl, as well as steroids devoid of sulfur, did not protect against acute $HgCl_2$ intoxication under our experimental conditions. Among a large series of organic and inorganic sulfur compounds, only dimercaprol (BAL) and Na-thioacetate exhibited any noteworthy antimercurial effect in acute tests. However, dimercaprol possesses considerable inherent toxicity whereas sodium thioacetate, though well tolerated in itself, frequently causes sudden death when given in combination with mercury. In view of these findings the thioacetylated steroids appear to represent a promising class of mercury antidotes.

It has been noted, 30 years ago, that following pretreatment with testosterone, mice become unusually resistant to the production of renal damage by $HgCl_2$. This effect had been ascribed to the well-known renotrophic action of anabolic androstane derivatives [6]. However, several years later it was found that under certain circumstances, cortisol (unlike desoxycorticosterone) offers similar protection although it is devoid of any renotrophic effect [17]. In both these instances the protective steroids had to be administered for several days prior to mercury poisoning, and even so, their prophylactic effect was manifest only against moderate doses of $HgCl_2$ which were not immediately fatal.

Independently of these investigations it became evident that, irrespective of their specific hormonal effects, certain steroids can also offer considerable protection against a great variety of intoxications with organic compounds [8]. All of these findings were essentially unplanned, chance observations made in the course of research on other subjects, without any knowledge of the underlying mechanisms that might be involved.

A more rational approach to this field was made possible only recently through the discovery in several other laboratories [1—4] that detoxicating enzymes can be induced in the hepatic microsomes by various drugs and hormones. It soon became evident that the previously noted nonspecific protective effect of some steroids is frequently due to the neoformation of such hepatic enzymes which can attack numerous substrates and thereby offer protection against a broad spectrum of noxious agents. For example, these "catatoxic steroids" were found

to inhibit the toxic effects of digitoxin [18], indomethacin [7, 12], phenindione [14], nicotine [19], various pesticides [16], barbiturates and steroid anesthetics [9], meprobamate [13], picrotoxin [15] and many other drugs [8]. In vitro observations suggest that the detoxication of most, if not all, of these drugs depends upon the induction by such steroids of drug-metabolizing enzymes in the hepatic microsomes [5, 20, 21].

In the case of catatoxic steroids, the necessity for several days of pretreatment is generally ascribed to the time required for the induction of drug-metabolizing hepatic enzymes. However, metabolic degradation could not explain the protection by testosterone or cortisol against inorganic mercury [6, 17]. Yet, recent preliminary observations showed that, spironolactone, one of the most potent catatoxic steroids, is far more effective than testosterone or cortisol in protecting against $HgCl_2$, even if administered only a few minutes before an LD_{100} amount of the latter [10].

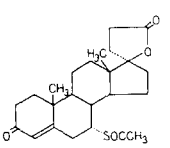
Many sulfur compounds are known to protect against mercury [22], hence, the question arose whether spironolactone owes this effect to its thioacetyl substituent. The experiments to be reported here were designed to clarify this point and to establish whether thioacetyl would be equally effective if unattached to a steroid and whether other sulfur compounds can offer similar protection when given alone or attached to various steroid molecules.

Materials and methods

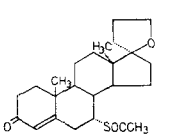
All our experiments were performed on female ARS/Sprague-Dawley rats divided into groups having a mean initial body weight of 100 g (range 90—110 g) and kept before and during the experiment on Purina Laboratory Chow.

The steroids tested were the following:

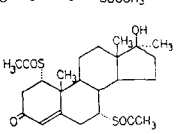
1. 17-Hydroxy-7α-thioacetyl-3-oxo-androstene 17α-propionic acid γ-lactone
   Spironolactone; Aldactone A^R; SC—9420 (Searle)



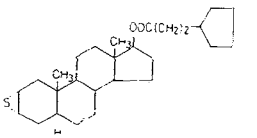
2. 7α-Thioacetyl-(17 R)-spiro-[4-androsten-17,2′-(furan)]-3-one
   Spiroxasone (Merck)



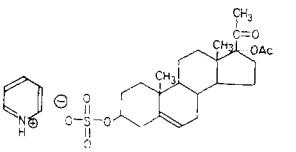
3. 17α-Methyl-17-hydroxy-1α,7α-dithio-4-androsten-3-one 1,7-diacetate
   Emdabol (Merck)



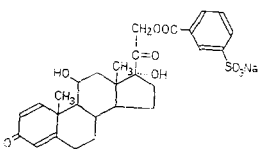
4. 2α,3α-Epithio-5α-androstan-17β-yl 3′-(cyclopentyl) propionate
SC-16179 (Searle)



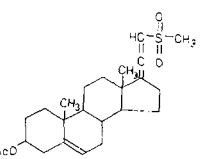
5. 3β,17-Dihydroxy-5-pregnen-20-one 3-pyridinium sulfate 17-acetate
AY-13,658—7 (Ayerst)



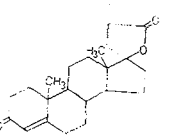
6. 11β,17,21-Trihydroxy-1,4-pregnadiene-3,20-dione 21-m-sodium sulfobenzoate
Prednisolone 21-m-sulfobenzoate sodium (Roussel)



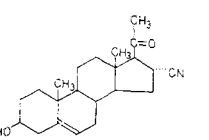
7. 3β-Acetoxy-5,17(20)-pregnatriene-21-methylsulfonate
SC-20719 (Searle)



8. 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl)-propanoic acid lactone
SC-5233 (Searle)



9. 3β-Hydroxy-20-oxo-5-pregnene-16α-carbonitrile
SC-4674 (Searle)



10. 17-Hydroxy-3-oxo-4,6-androstadiene-17α-propionic acid
Aldadiene; SC-9376 (Searle)
11. 17-Hydroxy-3-oxo-4,6-androstadiene-17α-propionic acid potassium salt
Aldadiene Kalium; SC-14266 (Searle)
12. 9α-Fluoro-11β,17-dihydroxy-3-oxo-4-androsterone-17α-propionic acid potassium salt
SC-11927 (Searle)
13. 17α-Ethyl-4-estren-17-ol
Ethylestrenol; Maxibolin® (Organon)
14. 13,17α-Diethyl-17-hydroxy-4-gonen-3-one
Norbolethone (Racemic); Genabol®; Wy-3475 (Wyeth)
15. 17α-Methyl-17-hydroxy-2-oxa-4-androsten-3-one
Oxandrolone (Searle)

16. 11β,17,21-Trihydroxy-1,4-pregnadiene-3,20-dione
Prednisolone acetate (Schering)
17. 9α-Fluoro-11β,16α-17,21-tetrahydroxy-1,4-pregnadiene-3,20-dione
Triamcinolone (Lederle)
18. 21-Hydroxy-4-pregnene-3,20-dione acetate
Desoxycorticosterone acetate (SKF)
19. 4-Pregnene-3,20-dione
Progesterone (Roussel)
20. 1,3,5(10)-Estratriene-3,17β-diol
Estradiol (Roussel)
21. 21-Hydroxy-5β-pregnane-3,20-dione hemisuccinate sodium salt
Hydroxydione sodium; Presuren®, Viadril® (Schering)

In the preceding list we indicated the structure formulas of the first nine compounds, because they are of special importance in connection with our topic. Cpd. 1—3 possess thioacetyl groups, Cpd. 4—7 contain sulfur in other forms, Cpd. 8 corresponds to the highly potent spironolactone (Cpd. 1) except that it is devoid of thioacetyl. Cpd. 9, though not previously tested against mercury, has proven to be the most potent among 304 steroids compared for their ability to detoxicate such organic substrates as digitoxin and indomethacin [11]. The other steroids were included in our study for comparative purposes. They contain no sulfur but are especially potent representatives of the antimineralocorticoid (Cpd. 10—12), anabolic (Cpd. 13—15), glucocorticoid (Cpd. 16, 17), mineralocorticoid (Cpd. 18), luteoid (Cpd. 19), folliculoid (Cpd. 20) and anesthetic (Cpd. 21) classes of steroids.

All these compounds were invariably administered as solutions or suspensions in 1 ml water by stomach tube at different dose levels.

Mercuric chloride ($HgCl_2$) was injected in 1 ml water into the jugular vein, under light ether anesthesia also at varying dose levels.

Irrespectively of the period of steroid pretreatment the surviving animals were invariably killed with chloroform on the fourth day after $HgCl_2$ administration and immediately submitted to autopsy. The degree of nephrocalcinosis was gauged (upon inspection with a dissecting loupe) in terms of an arbitrary scale in which 0 = no lesion, 1 = just detectable, 2 = moderate (usually only corticomedullary) and 3 = maximal (corticomedullary and cortical) calcification as previously described [10]. In addition, in dubious cases, specimens of renal tissue were fixed in alcohol-formol and stained with the Kossa technique. Entire specimens of some kidneys were defatted and then stained *in toto* with the Kossa procedure to bring out clearly the grossly visible blackened calcific deposits on the outer and cut surfaces (Figs 1—3).

Special technical details (dosage, timing, etc.) concerning this work with steroids, as well as the procedures used in the numerous comparative tests of non-steroidal sulfur compounds will be given in the descriptions of the individual experiments.

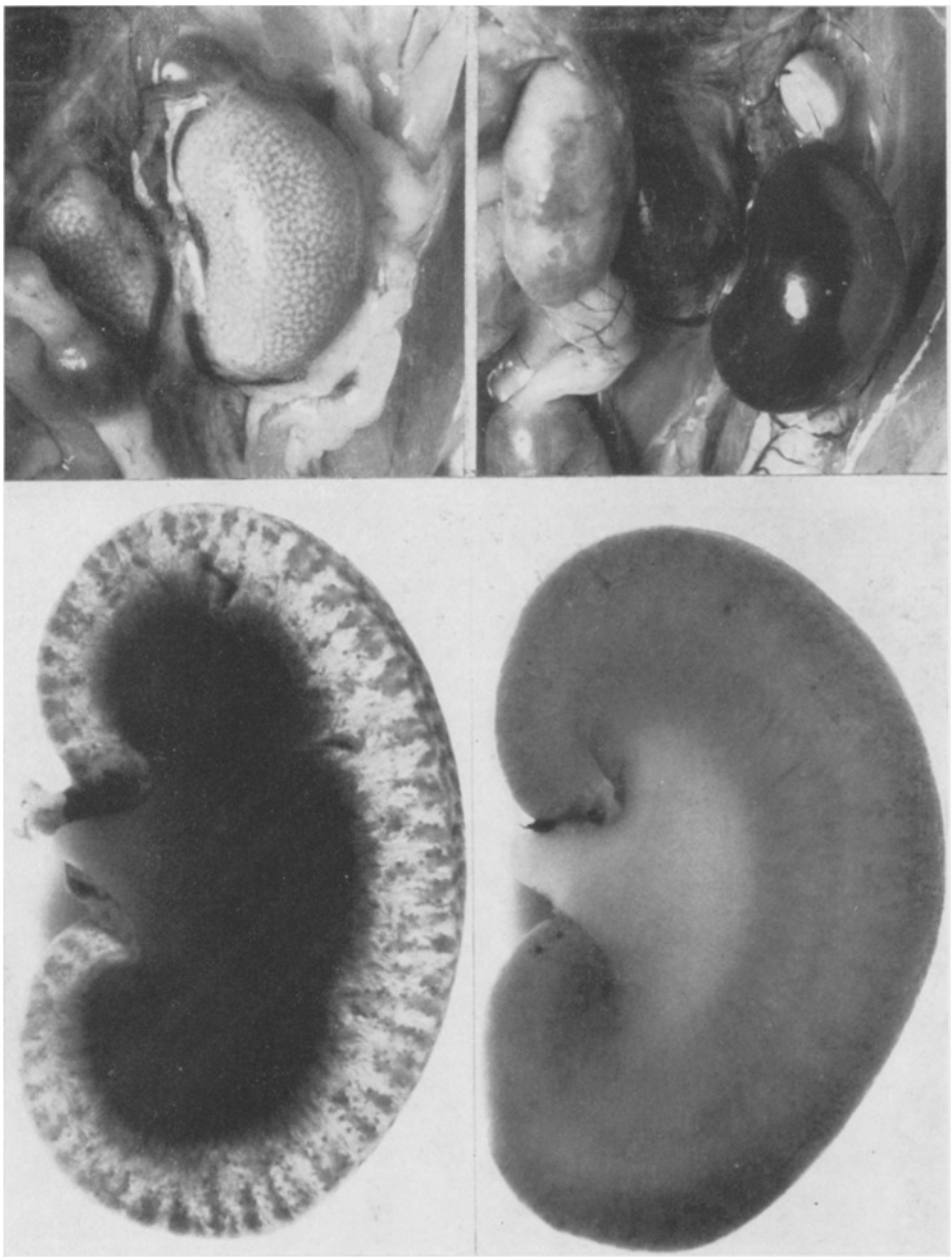


Fig. 1. Prevention of $HgCl_2$-induced nephrocalcinosis by spironolactone (fresh preparations). — Left: $HgCl_2$. Right: $HgCl_2$ + spironolactone. Top: Left kidney in its natural position with part of right kidney just visible across the mesentery of the rectum. Bottom: Cross section through the kidneys shown above. The tubular localization of calcification in the cortex after $HgCl_2$ alone is clearly visible as is its prevention by spironolactone

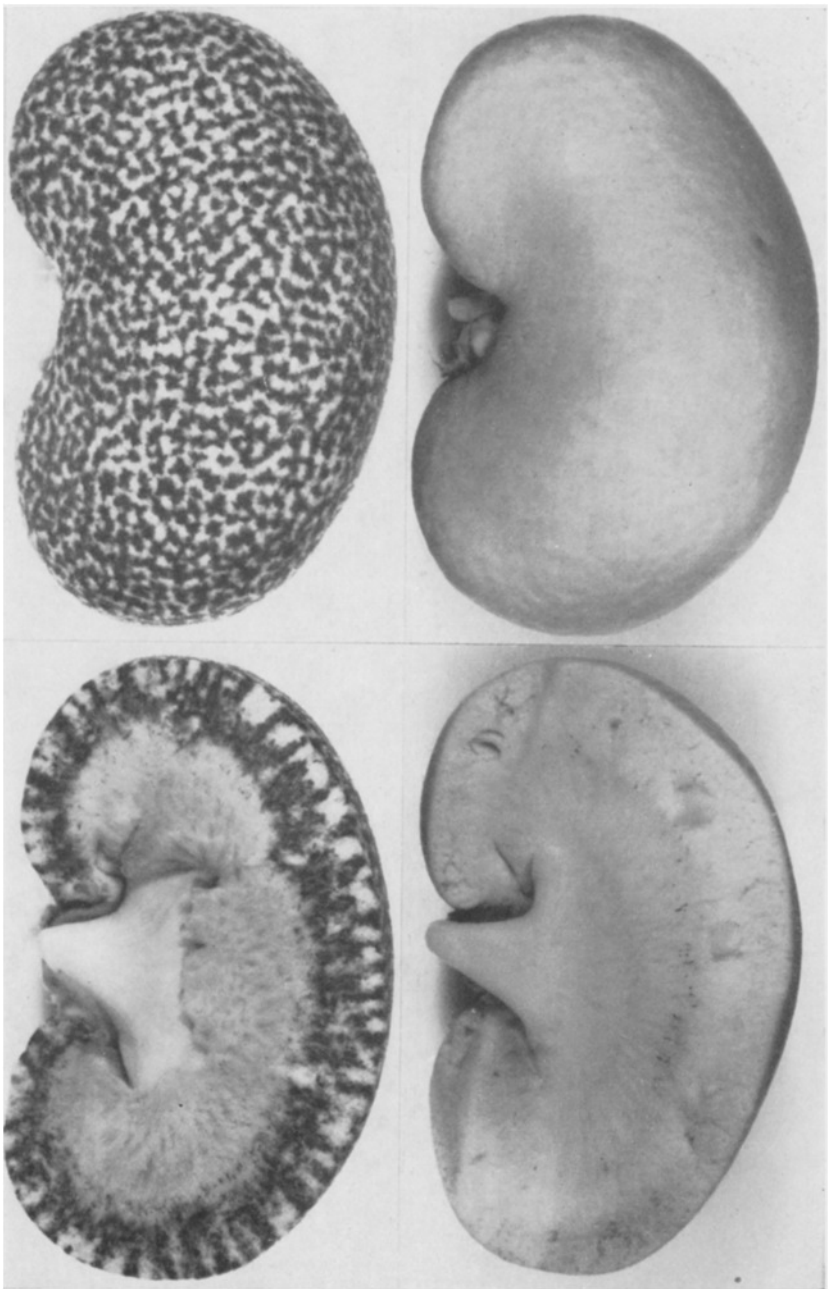


Fig. 2. Macroscopic appearance of the kidneys shown in Fig. 1 after staining of calcium salts. — Left: $HgCl_2$. Right: $HgCl_2$ + spironolactone. Top: External surface. Bottom: Cut surface. Note blackening of the mineral deposit with Kossa stain

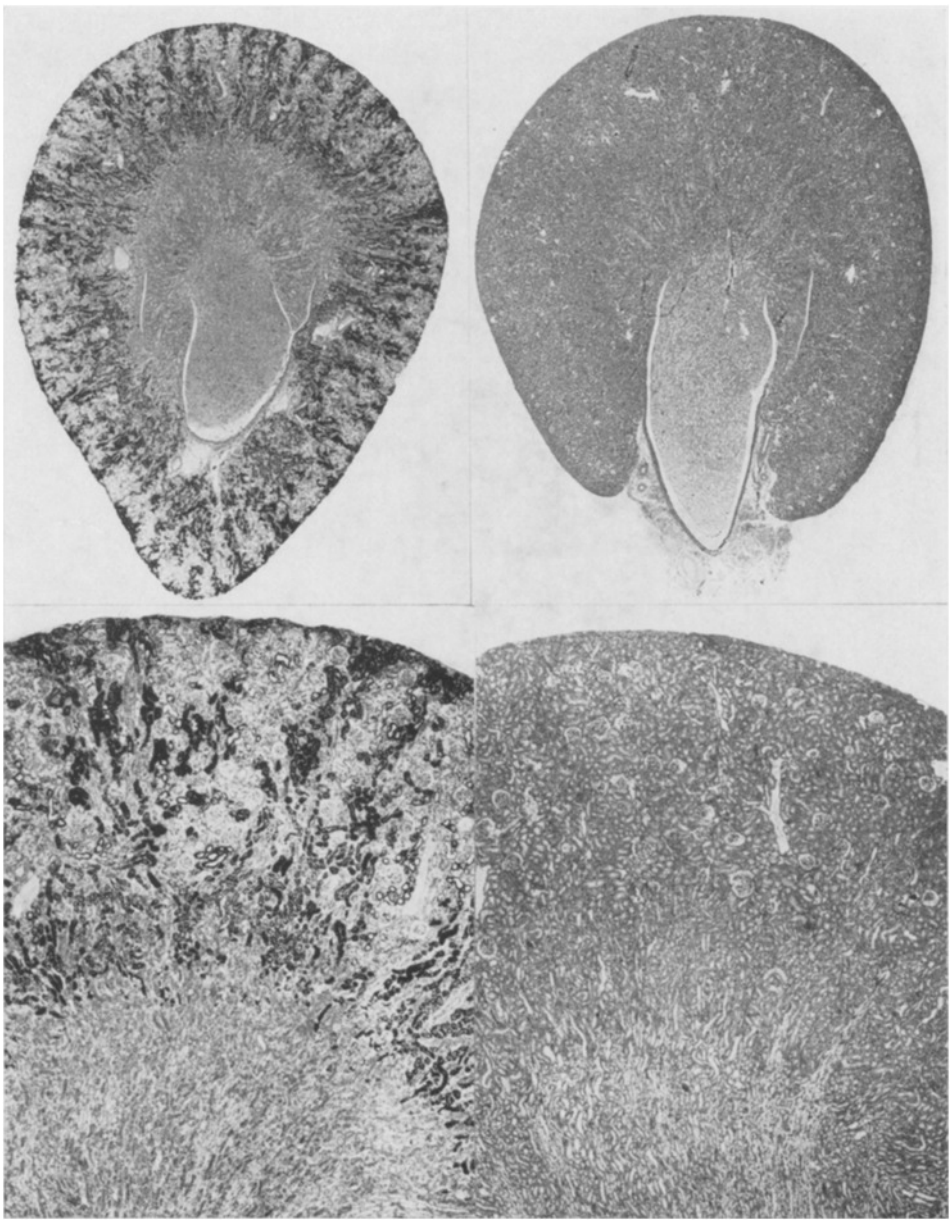


Fig. 3. Histological appearance of the kidneys shown in Figs 1 and 2. — Left: $HgCl_2$. Right: $HgCl_2$ + spironolactone. Top: Magnification ×11. Bottom: ×35 (Kossa stain). Mineralization is limited to certain segments of the cortical convoluted tubules. Following protection by spironolactone an essentially normal renal structure is maintained

Results

1. Protection against $HgCl_2$ by spironolactone given at different times. — The first preliminary experiment was designed to establish the optimal time of treatment for the prevention of $HgCl_2$ poisoning by spironolactone. For this purpose 12 groups of 5 rats each were given $HgCl_2$ (400 μg i.v.) preceded or followed by a single dose (100 mg p.o.) of spironolactone as indicated in Table 1.

Table 1

Protection against $HgCl_2$ by spironolactone given at different times

Group	Treatment*	Nephrocalcinosis (Scale: 0—3)	Mortality (%)
1	None	2.9	80
2	−48 hrs	3.0	100
3	−24 hrs	3.0	100
4	− 7 hrs	1.4	20
5	− 3 hrs	1.0	0
6	− 1 hr	0	0
7	−30 min	0	0
8	− 5 min	1.5	20
9	+ 1 min	3.0	80
10	+30 min	3.0	100
11	+ 1 hr	3.0	100
12	+ 7 hrs	3.0	100

* Spironolactone (10 mg p.o.) given at indicated times before or after $HgCl_2$ (400 μg i.v.) as described in the text.

It will be noted that this large amount of mercury caused virtually maximal nephrocalcinosis and a mortality of 80—100 % in the controls (Group 1) as well as in the rats which received the steroid as long as 48 or 24 hrs before (Groups 2 and 3) or 1 min. or more after (Groups 9—12) the mercury. All these experiments were terminated 4 days after injection of $HgCl_2$, hence the final mortality is not reflected by the data in the Tables. Numerous other experiments have shown that with a nephrocalcinosis of about 2.5 or more (in our scale) all animals die within about a week.

Some inhibition of $HgCl_2$ poisoning was noted among the rats given spironolactone 7 hrs, 3 hrs, or 5 min. before $HgCl_2$ (Groups 4, 5 and 8). Administration of the steroid 1 min. to 7 hrs after $HgCl_2$ (Groups 8—12) invariably failed to offer protection. On the other hand the rats receiving spironolactone 1 hr. or 30 min. before $HgCl_2$ (Groups 6 and 7) were completely protected as regards both nephrocalcinosis and mortality. Of course, since the steroid was administered p.o., some time lapse was to be expected before spironolactone could exert its beneficial effect against the instantly acting, i.v. administered, $HgCl_2$. On the basis

of these preliminary findings it was decided to administer potentially prophylactic agents 1 hr. before $HgCl_2$ in the subsequent experiments.

2. Protection against $HgCl_2$ by various doses of spironolactone. — In a second preliminary experiment we wanted to establish the minimum effective dose (MED) of spironolactone. For this purpose, 6 groups of 5 rats each were treated as indicated in Table 2.

Table 2

Protection against $HgCl_2$ by various doses of spironolactone

Group	Pretreatment*	Nephro-calcinosis (Scale: 0–3)	Mortality (%)
1	None	2.8	100
2	Spironolactone 10 mg	0	0
3	Spironolactone 5 mg	0.2	0
4	Spironolactone 2.5 mg	0.8	20
5	Spironolactone 1 mg	3.0	80
6	Spironolactone 0.5 mg	2.8	100

* One hour after the pretreatments listed in this column the rats of all groups received 400 μg of $HgCl_2$ as indicated in the text.

Table 3

Protection by spironolactone against various doses of $HgCl_2$

Group*	$HgCl_2$ (μg)	Control		Spironolactone	
		Nephro-calcinosis (Scale: 0–3)	Mortality (Dead/Total No)	Nephro-calcinosis (Scale: 0–3)	Mortality (Dead/Total No)
1–2	50	0	0/5	0	0/5
3–4	100	0.9	2/15	0	0/5
5–6	150	0.2	0/5	0	0/5
7–8	200	2.3	14/30	0	0/10
9–10	250	2.8	8/10	0	0/5
11–12	300	2.7	23/30	0	0/10
13–14	400	3.0	29/30	0	0/14
15–16	600	2.7	15/15	0.2	7/15
17–18**	1000	—	15/15	—	15/15

* The odd-numbered groups comprise controls given $HgCl_2$ alone, the even-numbered were pretreated with 10 mg of spironolactone 1 hr before receiving the same dose of $HgCl_2$, as described in the text.

** All animals treated with 1000 μg of $HgCl_2$ died too early for the appraisal of nephrocalcinosis.

Virtually complete protection was obtained by spironolactone at the dose of 10 and 5 mg (Groups 2 and 3), moderate protection at 2.5 mg (Group 4), whereas 1.0 and 0.5 mg (Groups 5 and 6) was ineffective.

3. Protection by spironolactone against various doses of $HgCl_2$. — The last preliminary experiment was designed to determine the limits of the protective effect offered by the highest previously tested dose of spironolactone given p.o. at the optimal time of 1 hr. before i.v. administration of $HgCl_2$. Under conditions otherwise identical to those of the previous two series, 18 groups of 5 to 30 rats were used for this purpose. The controls (odd numbered groups in Table 3) were given $HgCl_2$ alone, the others (even numbered groups in Table 3) received corresponding amount of $HgCl_2$ 60 min. after the standard amount of spironolactone.

As shown by Table 3, under these conditions, spironolactone offered perfect protection against both the nephrocalcinosis and the mortality elicited by $HgCl_2$ in doses up to 400 μg (Groups 1—14). Some protection against mortality, though not against nephrocalcinosis, was evident even after treatment with as much as 600 μg of $HgCl_2$ (Groups 13, 14). The rats treated with 1 mg of HgC_2l

Table 4

Effect of various steroids upon acute $HgCl_2$ poisoning

Group	Pretreatment*	Nephro-calcinosis (Scale: 0—3)	Mortality (%)
1	None	3.0	90
2	Spironolactone	0	0
3	Spiroxasone	0	0
4	Emdabol	0.1	0
5	SC—16 179	2.7	80
6	AY—13,658—7	2.8	90
7	Prednisolone sulfobenzoate	2.7	60
8	SC—20 719	2.7	70
9	SC—5233	3.0	60
10	SC—467	3.0	100
11	Aldadiene	2.9	90
12	Aldadiene Kalium	2.8	100
13	SC—11 927	3.0	80
14	Ethylestrenol	2.9	90
15	Norbolethone	3.0	80
16	Oxandrolone	2.9	90
17	Prednisolone acetate	2.6	100
18	Triamcinolone	2.3	50
19	DOC-acetate	2.9	60
20	Progesterone	2.8	90
21	Estradiol	3.0	80
22	Hydroxydione	2.9	100

* One hour after the pretreatment listed in this column the rats of all groups received 300 μg of $HgCl_2$ as indicated in the text.

all succumbed so soon that nephrocalcinosis could not yet have become evident (Groups 17, 18). Somewhat unexpectedly, the spironolactone treated rats given 600 μg or 1 mg of $HgCl_2$ showed severe dyspnoea and most of them died within the first few hours when the controls that received no spironolactone were still in good condition. In other words, here, spironolactone appears to have exerted an inverse effect, although at least after treatment with 600 μg of $HgCl_2$ it diminished the final mortality and virtually abolished nephrocalcinosis among the survivors. We shall have more to say about this singular inverse effect later.

4. Effect of various steroids upon acute $HgCl_2$ poisoning. — In the principal experiment 21 steroids were tested under the conditions shown — by the three preliminary series just described — to be optimal for the prevention of severe $HgCl_2$ intoxication. Here, 22 groups of 10 rats each were treated as outlined in Table 4. All steroids were administered at the dose of 10 mg in 1 ml water p.o. followed 1 hr. later by an i.v. injection of 300 μg of $HgCl_2$.

Spironolactone, spiroxasone and emdabol (Groups 2—4), all of which contain thioacetyl groups, offered excellent protection, whereas none of the other compounds was effective. Even the steroids containing sulfur, but in forms other than thioacetyl (Groups 5—8) were totally devoid of $HgCl_2$-antagonizing potency. Similar negative results were obtained with SC-5233 (spironolactone without thioacetyl) and the catatoxic steroids of this series (Groups 10—16), which had previously been shown to be highly potent in detoxicating various organic poisons.

5. Effect of various non-steroidal sulfur compounds upon acute $HgCl_2$ poisoning. — Having learned that several sulfur containing steroids possess considerable prophylactic potency against acute $HgCl_2$ poisoning, we wanted to test some non-

Table 5

Effect of various non-steroidal sulfur compounds upon acute $HgCl_2$ poisoning

Group	Pretreatment	Dose	Nephro-calcinosis (Scale: 0—3)	Mortality (Dead/Total No)
1	None	—	2.7	23/30
2	Na-Thioacetate	10 mg p.o.	0.1	4/12
3	Thioacetamide	10 mg p.o.	1.5	4/10
4	Glutathione	10 mg p.o.	2.4	8/10
5	L-Cysteine HCl	10 mg p.o.	2.6	8/10
6	L-Cystine	10 mg p.o.	2.4	10/10
7	dl-Penicillamine	20 mg i.p.	2.8	2/5
8	Thiamine HCl	20 mg p.o.	2.4	2/5
9	Biotin	20 mg p.o.	1.6	2/5
10	Taurine	20 mg p.o.	2.9	10/10
11	Dimercaprol (BAL) 3%	0.2 ml s.c.	0	1/10
12	$NaHSO_4$	1 mM s.c.	2.5	7/10
13	Na_2SO_4	1 mM s.c.	3.0	13/20
14	$Na_2S_2O_3$	1 mM s.c.	2.5	5/10
15	NH_4HSO_4	1 mM s.c.	2.8	10/10

steroidal sulfur compounds for comparison. For this purpose we selected both organic and inorganic substances which had been previously shown to be, or at least suspected of being, effective antidotes for mercury [22]. As shown in Table 5, all compounds of this series (except dimercaprol, which is too toxic) were administered in amounts containing considerably more sulfur than the fully effective dose (10 mg) of spironolactone, spiroxasone or emdabol.

To facilitate the detection of a protective effect, $HgCl_2$ was administered at the dose of 300 μg 1 hr. after pretreatment with the sulfur compounds, that is, under conditions in which our thioacetylated steroids were always optimally effective. Yet, it is evident from Table 5 that, with the exception of sodium thioacetate and dimercaprol, none of our non-steroidal sulfur compounds exhibited any noteworthy prophylactic action against this acute form of $HgCl_2$ intoxication. Because of its well-known toxicity, no further work was undertaken with dimercaprol whose value and limitations as a detoxicant for heavy metal poisoning are in any event well-known. However, further investigations on Na-thioacetate appeared to be indicated for comparison with the activity of the thioacetyl group when attached to steroids.

6. Protection against $HgCl_2$ by various doses of Na-thioacetate. — 18 groups of 5—25 rats each were treated as indicated in Table 6. Since Na-thioacetate

Table 6

Protection against $HgCl_2$ by various doses of Na-thioacetate

Group	$HgCl_2$ (μg)	Na-thioacetate (mg)	Time of pre-treatment (min)*	Nephro-calcinosis (Scale: 0—3)	Mortality (Dead/Total No)
1	400	0	60	2.8	10/10
2	400	10	60	—**	5/5
3	400	2.4	60	3.0	5/5
4	400	1.2	60	3.0	5/5
5	400	0.6	60	3.0	5/5
6	400	10	30	—**	5/5
7	400	2.4	30	2.5	12/15
8	400	1.2	30	2.6	8/10
9	400	0.6	30	2.8	10/10
10	300	0	60	3.0	5/5
11	300	10	60	0.1	8/25
12	300	2.4	60	2.6	5/5
13	300	1.2	60	3.0	5/5
14	300	0.6	60	3.0	5/5
15	300	10	30	0	12/15
16	300	2.4	30	1.4	0/5
17	300	1.2	30	2.6	2/5
18	300	0.6	30	3.0	5/5

* Time elapsed between pretreatment with Na-thioacetate p.o. and treatment with $HgCl_2$ i.v.

** All animals died too early for appraisal of nephrocalcinosis.

could not be commercially obtained we prepared it by neutralizing thioacetic acid with an equimolar amount of sodium bicarbonate. Various doses of this preparation were administered p.o. 30 or 60 min. before the i.v. administration of 400 or 300 μg of $HgCl_2$ as indicated in Table 6. Under none of these conditions did Na-thioacetate protect as well against $HgCl_2$ as did the thioacetylated steroids.

Several preliminary experiments had shown that even 20 mg of Na-thioacetate produces no detectable toxic effects in itself and in the present series 10 mg was very well tolerated by the animals of Group 2, 6, 11 and 15 until they were given $HgCl_2$. However, immediately after the administration of mercury, all these rats showed severe dyspnoea and most of them died within the next few hours with symptoms very similar to those previously mentioned in connection with the "inverse effect" of spironolactone when it is given in combination with very large amounts of $HgCl_2$ (cf. Table 3, Groups 15—18). However, Na-thioacetate produces this effect even in combination with smaller amounts of $HgCl_2$ against which spironolactone offers perfect protection. In the present series 10 mg of Na-thioacetate given in combination with 400 μg of $HgCl_2$ caused 100 % mortality whether the two treatments were separated by intervals of 60 min. (Group 2) or 30 min. (Group 6). However, some of the animals given 10 mg of Na-thioacetate + 300 μg of $HgCl_2$ survived (Groups 11 and 15) and in these, nephrocalcinosis was almost invariably absent.

Since 10 mg of Na-thioacetate contains much more thioacetyl than fully effective doses of spironolactone, emdabol or spiroxasone, it was felt that smaller doses might be effective without causing deaths. Hence, additional groups of rats were given Na-thioacetate in amounts of 2.4, 1.2 and 0.6 mg (equimolar respectively with 10, 5 and 2.5 mg of spironolactone) that is, doses corresponding in sulfur content to those in which the steroid had previously been shown to be efficacious (cf. Table 2, Groups 2, 3 and 4). Such small amounts of Na-thioacetate are generally devoid of immediate toxicity (the "inverse effect"), however, as Table 6 shows they also offer little, if any, protection against nephrocalcinosis or delayed death under any of the circumstance of dosage and timing.

Interestingly many of the rats receiving high doses of Na-thioacetate in combination with $HgCl_2$ — unlike those given the highest tested amounts of either of these substances alone — revealed partial or complete, usually bilateral, adrenocortical necroses.

Discussion

Numerous chelating agents and particularly dimercaprol have been shown to exert useful prophylactic effects against intoxication with various heavy metals including mercury. However, all of these compounds are quite toxic which makes it hazardous to administer the comparatively large amounts required in order to provide useful protection. By contrast, spironolactone is virtually devoid of toxic or undesirable hormonal effects in experimental animals at any dose level tested. The long experience with this drug, as an antimineralocorticoid in the

treatment of cardiovascular disease, has shown it to be well tolerated by man also. Essentially the same may be said about spiroxasone and emdabol. It is interesting therefore that all three of these thioacetylated steroids are powerful prophylactic agents against acute mercury intoxication.

Since none of the other sulfur containing steroids of our series offers any noteworthy protection against $HgCl_2$, the thioacetyl group as such appears to play a decisive role here. This view has received further support from the finding that spironolactone deprived of its thioacetyl group (that is, compound SC-5233) is inactive in this respect, whereas Na-thioacetate does possess a definite prophylactic effect, although only at dangerous dose levels. In amounts which protect against renal damage, Na-thioacetate produces considerable and almost immediate mortality in the presence of $HgCl_2$. The mechanism of this toxicity that results from conjoint treatment with a toxic metal and its potential antidote, remains to be elucidated. We have seen that in itself Na-thioacetate is well tolerated even at dose levels far above those which are lethal in the presence of mercury. On the other hand, amounts of $HgCl_2$ which invariably kill after a few days cause no immediate mortality in the absence of Na-thioacetate pretreatment. It is conceivable, though unproven, that Na-thioacetate forms a compound with mercury which is comparatively well tolerated unless excessive amounts of it flood the organism very suddenly. Assuming that the reaction product is very insoluble it may form precipitates which, if given time to be cleared by the RES, prevent acute mercurial intoxication, but if too rapidly formed in excessive amounts they may kill by obstructing important sectors of the microcirculation. The frequent occurrence of adrenal necroses in rats, otherwise protected against $HgCl_2$ by Na-thioacetate, might also result from such microemboli.

By contrast spironolactone reliably protects against normally fatal doses of $HgCl_2$. This steroid causes immediate mortality only after administration of several times the lethal dose of $HgCl_2$ and hence the safety margin of its prophylactic effect is much greater than that of Na-thioacetate. It is conceivable that when thioacetyl is bound to a steroid molecule, it is made available more gradually for combination with Hg than when the thioacetyl radical is given as the Na salt.

The structure of the steroid to which thioacetyl is bound, apparently plays a comparatively minor role here. The cyclic side-chain of spironolactone contains two oxygens whereas that of spiroxasone possesses only one and emdabol has a methyl group in position 17 instead of a cyclic side-chain, yet all three compounds are highly potent, nontoxic antagonists of $HgCl_2$.

In any event, our observations show that by binding thioacetyl to a steroid nucleus its antimercurial effect is greatly enhanced and its potential toxicity diminished.

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